

## REMARKS

Claims 1-14 are pending in this application.

### Claim Rejection - 35 U.S.C. §103(a)

Claims 1-14 have been rejected under 35 U.S.C. §103(a) as obvious over Lees (US Patent No. 5849301) in view of Penney et al. (US Patent No. 5773007) and Peetermans et al. (US Patent No. 6756040). To establish a *prima facie* case of obviousness, three basic criteria must be met: first, the prior art reference (or references when combined) must teach or suggest all the claim limitations; second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; finally, there must be a reasonable expectation of success. See M.P.E.P. § 2143. Lees, Penney et al. and Peetermans et al. do not teach or suggest all of the limitations of pending Claim 1 and its corresponding dependent Claims 2-14, and thus a *prima facie* case of obviousness cannot be established. Moreover, Lees teaches away from the invention. The totality of the prior art must be considered, and proceeding contrary to accepted wisdom in the art is evidence of nonobviousness. *In re Hedges*, 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986). A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

Pending Claim 1 recites “[a] method for preparing a conjugate vaccine, the method comprising: reacting a polysaccharide with an oxidizing agent, whereby a solution of an aldehyde-activated polysaccharide is obtained; reacting a protein with hydrazine dichloride at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained; reacting the aldehyde-activated polysaccharide with the hydrazine-activated protein at a pH of from about 5 to about 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained; and neutralizing unreacted aldehyde groups with adipic acid dihydrazide, whereby a conjugate vaccine capable of stimulating an immune response is obtained” (emphasis added). In the Office Action it is asserted that Lees teaches the highlighted step. Applicants respectfully disagree.

In the Background of the Invention section of Lees, various techniques for reacting and modifying carbohydrates, such as polysaccharides, are discussed. These include utilizing aldehyde groups on the polysaccharide; however, Lees teaches away from such techniques due to the slowness and low yields of such methods:

Coupling of proteins can also be achieved through reductive amination, either using the aldehyde found on the reducing end of the polysaccharide or created by oxidation of the carbohydrate. Both of these approaches have intrinsic limitations and, thus, for high molecular weight polysaccharides, coupling through the reducing end is usually slow and inefficient and oxidation often results in cleavage of the polysaccharide chain or otherwise affects the antigen. (Lees, col. 3, lines 22-25).

Other carbohydrates have aldehyde groups at the terminal reducing end that can be exploited for derivatization and conjugation. It is also possible to create aldehyde groups with oxidizing reagents, e.g., sodium periodate. Aldehyde groups can be condensed with amino groups on protein or with a bifunctional linker reagent. This condensation reaction, especially with the terminal reducing end of a high molecular weight polysaccharide, however, often proceeds quite slowly and inefficiently. This is exacerbated when directly conjugating carbohydrate aldehydes to proteins. Thus, yields are often very low using this method. Moreover, sodium periodate may break up carbohydrates into smaller fragments and/or disrupt epitopes, which may be undesirable. (Lees, col. 3, lines 42-55).

Recognizing the disadvantages of these prior art methods, Lees notes that:

Most carbohydrates must be activated before conjugation, and cyanogen bromide is frequently the activating agent of choice. ... To briefly summarize the CNBr-activation method, cyanogen bromide is reacted with the carbohydrate at a high pH, typically a pH of 10 to 12. At this high pH, cyanate esters are formed with the hydroxyl groups of the carbohydrate. These, in turn, are reacted with a bifunctional reagent, commonly a diamine or a dihydrazide. These derivatized carbohydrates may then be conjugated via the bifunctional group. In certain limited cases, the cyanate esters may also be directly reacted to protein. (Lees, col. 3, line 56 to col. 4, line 7).

However, this particular method utilizing cyanogen bromide suffers drawbacks too, as noted by Lees, including damage to the carbohydrate or protein components of the conjugate due to the high pH and instability of the cyanate ester at high pH, and difficulties in controlling the cyanogen bromide activation reaction. Lees also discusses modification of proteins by adipic

dihydrazide in the context of the cyanogen bromide method, but again, teaches away from such techniques due to excessive crosslinking and polymerization:

Limited derivatization of the protein by addition of a limited number of spacer groups, such as hexane diamine or adipic dihydrazide, has also been proposed. These may then be added, for example, by the cyanogen bromide method. In this method, protein carboxyls are activated with carbodiimides and reacted with the amine or hydrazide. This method, however, produces extensive crosslinking of protein and polysaccharide and polymerization of the protein. (Lees, col. 5, lines 15-22).

Lees' solution to the drawbacks of the cyanogen bromide method and other prior art methods discussed is to activate the carbohydrate with 1-cyano-4(dimethylamino)-pyridinium tetrafluoroborate (CDAP) at a pH of 6 to 10. The resulting CDAP-activated carbohydrate is then either directly conjugated to the protein, or conjugated to, e.g., a protein that has been derivatized with a hydrazine.

Lees neither teaches nor suggests reacting an aldehyde-activated polysaccharide with a hydrazine-activated protein at a pH of from about 5 to about 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained. To the contrary, Lees explicitly teaches away from conjugating an aldehyde-activated polysaccharide to a protein (see Lees at col. 3, lines 22-25 and lines 42-55). Lees also teaches that a high pH (10 to 12) is necessary for use in methods wherein cyanate ion (CN<sup>-</sup>) is generated (i.e., the cyanogen bromide activation method).

Applicants have discovered, despite teachings to the contrary in Lees and the other cited references and prior art, that aldehyde-activated polysaccharides can be employed as a reactant in a conjugation reaction to yield conjugate in high yield in a rapid reaction. Applicants have further discovered that it is not necessary to first activate a polysaccharide with CDAP in order to conduct a conjugation reaction at low pH, nor is it necessary to expose the polysaccharide to a high pH if cyanate generated is present. Applicants have surprisingly found that an aldehyde-activated polysaccharide can be conjugated to a protein in the presence of sodium cyanoborohydride – an inorganic cyanylating agent that generates cyanate ions – at low pH (5 to 7) when the protein has first been activated with hydrazide. This particular reaction is neither taught nor suggested by Lees.

Neither Penney et al. nor Peetermans et al. include teachings overcoming the deficiencies of Lees. Penney et al. teaches conjugation of an oxidized polysaccharide fragment directly to

tetanus toxoid monomer in the presence of sodium cyanoborohydride, but does not teach or suggest a hydrazide-modified protein. The only disclosure of Penney et al. regarding a hydrazide is a mention of the use of a symmetric linker such as adipic acid dihydrazide as described by Schneerson et al., J. Experimental Medicine, 152, 361-376 (1980). As noted in Applicants' application as filed, the Schneerson method is a cyanogen bromide conjugation method wherein the polysaccharide was subjected to a high pH of 10.5 (see Schneerson et al., page 363, paragraph entitled "HIB PS-Protein Conjugates"). Peetermans et al. includes similar disclosures as Penney et al., namely a description of cyanogen bromide coupling as described in Chu et al., Infect Immun. 1983 April; 40(1): 245-256. Again, the polysaccharide was subjected to a high pH of 10.5 (see Chu et al., page 247, first column, third full paragraph). Accordingly, the disclosures of Penney et al. and Peetermans et al. are therefore cumulative to the disclosures of Lees.

Because a method for preparing a conjugate vaccine comprising, *inter alia*, reacting an aldehyde-activated polysaccharide with a hydrazine-activated protein at a pH of from about 5 to about 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained is neither taught nor suggested by Lees, Penney et al. and Peetermans et al., alone or in combination, and is, in fact, taught away from by Lees, a *prima facie* case of obviousness cannot be established. Applicants therefore respectfully request withdrawal of the rejection.

*No Disclaimers or Disavowals*

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, the Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

**Application No.:** 10/566,898  
**Filing Date:** October 26, 2006

Co-Pending Applications of Assignee

Applicant wishes to draw the Examiner's attention to the following co-pending application of the present application's assignee.

Serial Number	Title	Filed
10/566899	Polysaccharide-Protein Conjugate Vaccines	September 25, 2006

Conclusion

In view of the foregoing, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns that might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: 1/17/08

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AMEND

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